

WHAT IS CLAIMED IS:

- 1 1. An isolated enone reductase having the physicochemical properties of (A)-
2 (C):
3 (A) it reduces the carbon-carbon double bond of an α,β -unsaturated ketone, using
4 NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;
5 (B) it has a substrate specificity of (1)-(4):
6 (1) it has substantially no activity to reduce the keto group of a ketone;
7 (2) it exhibits a significantly higher activity with NADPH than with
8 NADH as an electron donor;
9 (3) it does not substantially act on substrates wherein both substituents at
10 the β carbon relative to the ketone are not hydrogen; and
11 (4) it does not substantially act on a substrate in which the carbon-carbon
12 double bond is present in a cyclic structure; and
13 (C) it has an optimal pH of 6.5-7.0.
- 1 2. The enone reductase of claim 1, wherein the reductase (a) has an optimum
2 temperature of 37-45°C; and (b) has a molecular weight determined by sodium dodecyl
3 sulfate-polyacrylamide gel electrophoresis and by gel filtration of about 43,000 and about
4 42,000, respectively.
- 1 3. The enone reductase of claim 1, which is derived from an organism of the
2 genus *Kluyveromyces*.
- 1 4. A method for obtaining an enone reductase, comprising the step of
2 (a) culturing a microorganism belonging to the genus *Kluyveromyces*; and (b) isolating the
3 enone reductase of claim 1 from the cultured microorganism.
- 1 5. The method of claim 4, wherein the microorganism belonging to the genus
2 *Kluyveromyces* is *Kluyveromyces lactis*.
- 1 6. An isolated nucleic acid of any one of (a) to (d) below:
2 (a) a nucleic acid encoding a protein comprising the amino acid sequence of
3 SEQ ID NO:2;

4 (b) a nucleic acid comprising a coding region of the nucleotide sequence of
5 SEQ ID NO:1;

6 (c) a nucleic acid encoding a protein that comprises the amino acid sequence
7 of SEQ ID NO: 2, in which one or more amino acids are substituted, deleted, inserted and/or
8 added and that is functionally equivalent to a protein consisting of the amino acid sequence
9 of SEQ ID NO: 2;

10 (d) a nucleic acid that hybridizes under stringent conditions with a nucleic acid
11 consisting of the nucleotide sequence of SEQ ID NO: 1, and that encodes a protein
12 functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:2;
13 and

14 (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid
15 sequence of SEQ ID NO:2.

1 7. An isolated nucleic acid encoding the amino acid sequence of SEQ ID NO:2
2 or a fragment thereof.

1 8. A vector comprising the nucleic acid of claim 6.

1 9. A vector comprising the nucleic acid of claim 7.

1 10. The vector of claim 8, further comprising a nucleic acid sequence encoding a
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

1 11. The vector of claim 9, further comprising a nucleic acid sequence encoding a
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

1 12. A transformant harboring the nucleic acid of claim 6.

1 13. A transformant harboring the nucleic acid of claim 7.

1 14. A transformant harboring the vector of claim 8.

1 15. A transformant harboring the vector of claim 10.

1 16. A substantially purified polypeptide encoded by the nucleic acid of claim 6.

1 17. A substantially purified polypeptide encoded by the nucleic acid of claim 7.

1 18. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 12 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.

1 19. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 13 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.

1 20. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 14 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.

1 21. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 15 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.

1 22. An isolated nucleic acid of any one of (a) to (d) below:

2 (a) a nucleic acid encoding a protein comprising the amino acid sequence of
3 SEQ ID NO:4, 6 or 8;

4 (b) a nucleic acid comprising a coding region of the nucleotide sequence of
5 SEQ ID NO:3, 5 or 7;

6 (c) a nucleic acid encoding a protein that comprises the amino acid sequence
7 of SEQ ID NO:4, 6 or 8 in which one or more amino acids are substituted, deleted, inserted
8 and/or added and that is functionally equivalent to a protein consisting of the amino acid
9 sequence of SEQ ID NO:4, 6 or 8;

10 (d) a nucleic acid that hybridizes under stringent conditions with the nucleic acid
11 consisting of the nucleotide sequence of SEQ ID NO: 3, 5 or 7, and that encodes a protein
12 functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4, 6
13 or 8; and

14 (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid
15 sequence of SEQ ID NO:4, 6 or 8.

- 1 23. A substantially purified polypeptide encoded by the nucleic acid of claim 22.
- 1 24. A vector comprising the nucleic acid of claim 22.
- 1 25. The vector of claim 24, further comprising a nucleic acid sequence encoding a
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.
- 1 26. A transformant harboring the nucleic acid of claim 2.
- 1 27. A transformant harboring the vector of claim 24.
- 1 28. A transformant harboring the vector of claim 25.
- 1 29. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 26 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.
- 1 30. A method for producing a polypeptide, the method comprising the steps of
2 culturing the transformant of claim 27 and recovering a polypeptide expressed from the
3 transformant or the culture supernatant thereof.
- 1 31. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with the
3 enone reductase of claim 1.
- 1 32. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with the
3 polypeptide of claim 16.
- 1 33. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with the
3 polypeptide of claim 17.
- 1 34. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with the
3 polypeptide of claim 23.

1 35. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with a
3 microorganism that produces an enone reductase having the physicochemical properties of
4 (A)-(C):

5 (A) it reduces the carbon-carbon double bond of an α,β -unsaturated ketone, using
6 NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;

7 (B) it has a substrate specificity of (1)-(4):

8 (1) it has substantially no activity to reduce the keto group of a ketone;

9 (2) it exhibits a significantly higher activity with NADPH than with
10 NADH as an electron donor;

11 (3) it does not substantially act on substrates wherein both substituents at
12 the β carbon relative to the ketone are not hydrogen; and

13 (4) it does not substantially act on a substrate in which the carbon-carbon
14 double bond is present in a cyclic structure; and

15 (C) it has an optimal pH of 6.5-7.0.

1 36. The method of claim 35, wherein the microorganism is of the genus
2 *Kluyveromyces*.

1 37. The method of claim 35, wherein the microorganism is the transformant of
2 claim 12.

1 38. The method of claim 35, wherein the microorganism is the transformant of
2 claim 26.

1 39. A method for selectively reducing the carbon-carbon double bond of an
2 α,β -unsaturated ketone, comprising the step of reacting an α,β -unsaturated ketone with a
3 processed product of a microorganism that produces an enone reductase having the
4 physicochemical properties of (A)-(C):

5 (A) it reduces the carbon-carbon double bond of an α,β -unsaturated ketone, using
6 NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;

7 (B) it has a substrate specificity of (1)-(4):

8 (1) it has substantially no activity to reduce the keto group of a ketone;

- 9 (2) it exhibits a significantly higher activity with NADPH than with
10 NADH as an electron donor;
11 (3) it does not substantially act on substrates wherein both substituents at
12 the β carbon relative to the ketone are not hydrogen; and
13 (4) it does not substantially act on a substrate in which the carbon-carbon
14 double bond is present in a cyclic structure; and
15 (C) it has an optimal pH of 6.5-7.0.

1 40. The method of claim 38, wherein the microorganism is of the genus
2 *Kluyveromyces*.

1 41. The method of claim 38, wherein the microorganism is the transformant of
2 claim 12.

1 42. The method of claim 38, wherein the microorganism is the transformant of
2 claim 26.